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An investigation of the disinfection effect in joint action of the nanosecond electron beam and plasma radiation

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Abstract. The subject of the investigation of the possible synergy sterilizing effect included the joint action by the nanosecond electron beam (NEB) and plasma radiation of nanosecond gas discharge of high pressure. Plasma radiation was carried out by the GVI-150 generator which loading involved a discharge camera. Pulse repetition rate of the generator operation was 37 pps, and distance from electrodes cutoff to processed samples ~5 cm. The experiments on irradiation by the NEB were made on the pulse repetitive nanosecond accelerator URT-0.5 (electrons energy up to 500 keV, a pulse duration 50 ns, pulse repetition rate up to 200 pps). The time the vessel has been irradiated changed from 0 to 5 minutes; the absorbed dose (AD) of different batches changed from 0 to 5 kGy. The extensive data array on joint action of the NEB and plasma radiation for several types of widespread microorganism is received. Also, the synergetic effect of the NEB influence and plasma radiation on microorganisms such as *Klebsiella* is found.

1. Introduction

Plasma radiation of the gas discharge (PRGD) contains Ultraviolet radiation (UVR). A part of DNA are the pyrimidine bases, thymine and cytosine, differing in high photochemical activity in the field of 250–280 nm, are formed under the influence of UVR "stitchings" (dimers) which makes it impossible for DNA/RNA to double and reproduction to occur. The microorganism is inactivated thus doesn't constitute any danger to other "live" organisms any more. At the same time UVR doses are necessary for an inactivation of various pathogenic microorganisms including viruses, which differ slightly.

An alternative is radiation sterilization due to the universality of the striking influence of ionizing radiation on any biological objects. At the same time, the absorbed dose (AD) of radiation sterilization doesn't exceed 25 kGy.

However, at radiation of food various chemical reactions are possible which can change the properties of products that force to establish a limit AD at radiation of various products. For example, for a fresh egg the recommended AD level ≤ 3 kGy that is close to the AD level for an inactivation of bacteria in the *Salmonella* group [1]. The irradiated products are marked by the special sign "radur"



that a buyer could choose. Unfortunately, the radio phobia has serious value at the choice of consumers.

Therefore, the task of achieving the decreased AD which was due to the joint influence of PRGD of high pressure and the nanosecond electron beam (NEB) from various AD, in fact, was the purpose of this work.

2. Experimental

Beforehand, the samples were irradiated only by PRGD and then jointly by PRGD and the NEB.

Irradiation by PRGD were carried out on the GVI-150 [2] generator which loading was a discharge camera. The external electrode of the camera was the rust-resistant pipe with the diameter 60 mm (a wall 0.75mm), an inner electrode with the positive polarity such as an aluminum wire with a diameter 2 mm. The length of electrodes is 300 mm. with the pulse repetition rate 37 pps and distance from electrodes cutoff of electrodes to processed samples ~5 cm.

Table eggs were used so that bactericidal action of PRGD on eggs microflora could be studied. 10 eggs were placed in each plastic container. On the feed conveyor, the containers were passed through the work space of installation (by a cutoff of electrodes), being irradiated by PRGD. In order to avoid losses of PRGD in material, the container was not covered. After processing, the containers were turned over, so the eggs could be irradiated by PRGD on the other side. The time the container was under irradiation was equal to 3.5 s (time of egg irradiation ~0.7s). The energy enclosed in discharge ~375 dzh/container was not enough for sterilization, but it allowed to estimate efficiency of the method.

The experiments were executed with the help of the accelerator URT-0.5 [3] (electron energy up to 500 keV, pulse width ~50 ns, pulse repetition rate up to 200 pps) while the AD was defined by means of a film dosimeter SO AD(F)R-5/50. The method of irradiation and measurement of AD is described in [4]. In the course of the experiments, the accelerator performed under the voltage charge of 30 kV. On the feed conveyor, the closed plastic containers with 10 eggs were passed through the work space of installation evenly irradiated by NEB.

In order to search synergy effect, at first, batches of eggs were irradiated by PRGD and then by NEB. The container went under irradiation by PRGD 0, 1, 2 and 5 min., AD NEB changed with a step 0, 1, 3, and 5 kGy (Tab. 5). For producing AD, the accelerator worked at the following frequencies: 1 kGy – 3 Hz; 3 kGy – 10 Hz; 5 kGy – 30 Hz. The size of a batch was 20 eggs.

After irradiation took place, washouts from a surface of eggs were taken which subjected to a microbiological research; crops were carried out on nutrient mediums with allocation of cultures and identification of the received microorganisms. Table eggs were studied for 25 days, the term of the regulated storage at a temperature 0-20°C and humidity 85-88%. On the 12th and 25th day, the second and third microbiological control of pilot and control batches were made: washout from a surface of eggs with the subsequent crops on nutrient mediums (MPA, MPB), a thermostat control, allocation of pure cultures and identification of microorganisms. Besides the key chemical indicators of food, the eggs were investigated which method is described in detail in [5].

For researching the NEB action and PRGD and NEB joint action, standard strains of Salmonella were used. The crops were carried out on dense nutrient medium (Endo's circle) from cultivation of 5 billion microbic cells on 1 ml. Right after that, petri dishes were subjected to a nanosecond electron beam with the absorbed doses 1, 2, 3, 5 kGy. The experiment was performed in 3 parallels, control tests were in the same conditions as the experiment at hand, but they weren't processed by NEB. After irradiation the dishes placed in the thermostat and incubated at the temperature 35-37°C within 24 hours. Further on, the calculation of a number of colonies forming unit (CFU) were performed. The method of the analysis is described in detail in [5]. Results of experiments are given in tables 4 and 5.

3. Results and discussion

The results of the experiments are given in tables 1-5. From the table 1 it is seen that processing by PRGD reduces the general microbic content and leads to elimination of mushrooms such as *Aspergillus* spp.

It was experimentally established that after having tested the eggs irradiated by PRGD, the mass fraction of crude fat practically didn't differ ($10.8 \pm 0.52\%$) from control ($10.3 \pm 0.51\%$). The analysis of the content of albumin in eggs hasn't revealed statistically reliable changes and differences between tests. The research of amino acids revealed that the maintenance of lysine is a subject to the greatest changes made in the experiment at hand ($0.84 \pm 0.03\%$) and in control ($0.88 \pm 0.05\%$). All of the changes of qualities and properties of a shell as well as its internal structures revealed at macroscopic assessment correspond to natural changes at long storage. Consequently, the images and the obtained data (tables 2 and 3) confirm the lack of reliable differences between changes in the eggs processed by PRGD and in tests from control batch at storage.

Table 1. Microbiological evaluation of table egg processing efficiency by the PRGD.

Microorganism	Control, CFU/g			Experiment, CFU/g		
	1 st day	12 th days	25 th days	1 st day	12 th days	25 th days
<i>St.aureus</i>	520	4 300	7 200	20	120	460
<i>Aspergillus</i> spp.	10	90	300	no growth	no growth	no growth

During the radiation NEB, the culture of *Sal. Enteritidis* has shown dose-dependent effect (table 4). The quantity of cells decreases with growth of AD. At AD 3 kGy there is a considerable oppression of growth above; while at AD 5 kGy, growth was absent.

The result showed that the processing of table 2 eggs in commercial plastic package with a NEB suppresses the growth of microflora on the surface of eggs completely with an AD of 5 kGy and more. If the storage conditions are observed, the surface of the eggs remains a sterile one throughout the whole regulated shelf life. The growth of *St. aureus* was noted in control samples.

Microbiological analysis of swabs from the hatchery egg surface of the experimental batch revealed a complete absence of microorganism growth.

Table 2. Content of vitamins in protein before (control) and after processing by PRGD.

Kind of analysis	Vitamins content ME (mg/kg)	Egg control ME (mg/kg)
Vitamin A in yolk	10351 ± 490 (3.10 ± 0.15)	7647 ± 310 (2.79 ± 0.10)
Vitamin B ₂ in protein	(2.28 ± 0.11)	(7.06 ± 0.31)

Table 3. Content of elements in a shell before (control) and after processing by PRGD.

Kind of analysis	Content, mg/kg	Control, mg/kg
Calcium	366112 ± 18310	368192 ± 18399
Sodium	1531	908

On the culture of *Sal. Typhimurium* the results are more contradictory. There is a tendency of quantity of cells to decrease with the increase of a dose, but at 5 kGy the dependence is broken. Perhaps, the stimulating effect of small doses of irradiation or an error of an experiment takes place here. In general, it is possible to assume that this culture is steadier against irradiation by NEB.

Table 4. Results of processing Salmonella culture by the NEB method.

Microorganism	AD, kGy (measured)	Cell number, CFU/g	Note
Sal. Enteritidis	0	1000	More than 1000
	1.5	500	
	2	500	
	3	250	
	4.5	0	
Sal. Typhimurium	0	1000	More than 1000
	0.5	400	
	1.5	260	
	3	250	
	4	500	
Sal. Gallinarum	0	1000	More than 1000
	0.5	500	
	1	500	
	3	100	On the periphery
	4.5	100	On the periphery

Considering the fact that Salmonella Typhimurium is an activator of a paratyphoid, its low radio sensitivity assumes more careful control at radiation sterilization of food and medical tools.

Moreover, oppression of the growth of the culture of Sal. Gallinarum is observed with the increase of AD. However, the bactericidal effect is at 5 kGy it is still not full. Also, after the quantity of cells has decreased, full elimination didn't happen.

Table 5. Microorganism survival rate by joint action of NEB and PRGD methods.

		AD, kGy			
		0	1	3	5
PRGD, min.	0	Pseudomonas aeruginosa, Klebsiella spp., Enterococcus, Staphylococcus	Pseudomonas aeruginosa, Enterococcus, Staphylococcus	Klebsiella spp., Enterococcus, Staphylococcus	Staphylococcus
	1	Pseudomonas aeruginosa, Klebsiella spp., Staphylococcus	Klebsiella spp., Enterococcus, Staphylococcus	Klebsiella spp., Staphylococcus	Enterococcus, Staphylococcus
	2	Pseudomonas aeruginosa, Enterococcus, Staphylococcus	Pseudomonas aeruginosa, Klebsiella spp., Enterococcus, Staphylococcus	Enterococcus Staphylococcus	Enterococcus, Staphylococcus
	5	Klebsiella spp, Enterococcus, Staphylococcus	Pseudomonas aeruginosa, Klebsiella spp., Enterococcus, Staphylococcus	Enterococcus, Staphylococcus	Enterococcus, Staphylococcus

In the tests processed by NEB with AD 3 kGy suppression of growth of Pseudomonas aeruginosa is noted, and at the absorbed dose 3-5 kGy the Pseudomonas aeruginosa in washouts wasn't sowed. There was no influence of PRGD on this microorganism.

The growth of microorganisms such as Staphylococcus was observed in all tests that indicates their low radio sensitivity. At small doses the NEB with influence of PRGD or without it, oppression of growth isn't established. In this range of doses and time staphylococcus do well. The bactericidal effect is possible at higher doses (at 10-15 kGy).

However, for microorganisms such as Klebsiella, the synergetic effect of action the NEB and plasmas is found: at AD NEB 3 kGy these microorganisms are sowed when processing by plasma 0-1 minute, and at 3-5 minutes perish – in these tests growth isn't present (table 5).

Even though microorganisms of Enterococcus were sowed in all tests, oppressions of growth under action the NEB or plasmas isn't revealed.

4. Conclusion

The acquired results allow to draw a conclusion that at irradiation by PRGD the enclosed energy ~375 J is not enough for sterilization; however, it allows to estimate efficiency of the method as a perspective.

It is established that there are some tendencies at joint processing the NEB and PRVD radiation. However, there are no definitive results for different types of microorganisms.

Influence of the NEB in this range AD has boundary character; for example, a part of microorganisms perishes, and there is a part that doesn't. Perhaps, the stimulating effect of small doses of radiation takes place.

Therefore, microorganisms such as Salmonella and Pseudomonas aeruginosa are sensitive to the NEB in AD ~3 kGy. Microorganisms of Enterococcus and Staphylococcus are steadier against radiation which requires AD ~5 kGy for their destruction. Klebsiella is the only microorganism on which the synergetic effect of influence the NEB and the PRGD is established. The received results have more theoretical value which demand further development.

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